REMARKS

This paper is filed in response to the official action dated December 24, 2008 (hereafter, the "official action"). This paper is timely filed as it is accompanied by a petition for extension of time and authorization to charge our credit card account in the amount of the requisite fee. The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed, or which should have been filed herewith, to our Deposit Account No. 13-2855, under Order No. 30105/32001A.

Claims 11-27 are pending, but claims 25-27 have been withdrawn.

CLAIM REJECTIONS – 35 U.S.C. §103(a)

Claims 11-13 have been rejected as assertedly obvious over Kaddurah-Daouk et al. (WO 97/13507A1) and Wessel et al. (U.S. Patent No. 5,948,810). Claim 14 has been rejected over the combination of Kaddurah-Daouk et al. and Wessel et al. in further view of Smith et al. (U.S. Patent No. 5,039,704).

Claims 15-24 have been rejected as assertedly obvious over the combination of Gardiner (U.S. Patent No. 5,817,329), Brantman (U.S. Patent No. 4,687,782), Michnowski (U.S. Patent No. 4,832,971), Riley (U.S. Patent No. 5,976,568), Ecker (U.S. Patent No. 3,894,148), Product Alert (1996), Majeed et al. (U.S. Patent No. 5,536,506), and Pariza et al. (U.S. Patent No. 5,856,149).

The rejections are respectfully traversed.

Claims 11-14

Kaddurah-Daouk et al. teach that creatine is useful for the treatment of a glucose metabolic disorder such as diabetes mellitus. More specifically, Kaddurah-Daouk et al. teach that at least two creatine compounds, creatine phosphate and cyclocreatine, are hypoglycemic agents and cause glucose levels to drop significantly in a subject (page 4, lines 20-22).

Wessel et al. teach that (R)-(+)-alpha lipoic acid is useful for the treatment of diabetes mellitus. Wessel et al. further teach that "(S)-(-)-alpha lipoic acid practically is not usable" for the treatment of diabetes mellitus (column 2, lines 34-38). In fact, Wessel et al. teach that administration of (S)-(-)-alpha lipoic acid actually counteracts the benefits attained using (R)-(+)-alpha lipoic acid (column 2, lines 38-41; column 4, lines 1-2; column 4, lines 40-42). In view of this explicit teaching, there would be no

motivation to employ the combination of lipoic acid (a racemic mixture) and creatine, as claimed, because one cannot presume the alpha lipoic acid racemate has the same biological activity as the (R)-(+)-alpha lipoic acid enantiomer identified by Wessel et al. as being useful for the treatment of diabetes mellitus. Accordingly, a *prima facie* case of obviousness cannot be sustained and the rejection of claim 14 should be removed for this additional reason.

Moreover, the reasoning advanced relative to claim 14 is defective for at least the following additional reason. Smith et al. teach that a catabolic dysfunction can be treated by administering a therapeutically effective amount of glutamine (column 3, lines 47-49), and that parenteral catabolic dysfunctions which display increased demand for glutamine occur during uncontrolled diabetes (column 5, lines 19-22). According to Stumvoll et al., *Kidney International* 55:778–792 (1999) "Glutamine is an important glucose precursor and makes a significant contribution to the addition of new carbon to the glucose carbon pool" (abstract).[1] Thus, *administration of glutamine actually increases glucose levels*. Accordingly, glutamine does not have the same biological activity as creatine, which Kaddurah-Daouk et al. teach is a hypoglycemic agent that causes a decrease in glucose levels. In view of the foregoing, the applicants respectfully submit that one of ordinary skill would not be motivated to add a component to a mixture when that component performs an opposing function to another component in the mixture. There would be no reasonable expectation of success in formulating an antidiabetic composition when two components work against one other. Accordingly, a *prima facie* case of obviousness cannot be sustained and the rejection of claim 14 should be removed for this additional reason.

Claims 15-24

The Office states that "it would have been obvious to one of ordinary skill in the art to modify Gardiner's nutritional food supplement and incorporate other agents such as fructose, soy protein isolate, l-carnitine, grape seed extract, coenzyme Q10, piper nigrum extract, alpha lipoic acid, l-leucine, l-alanine and glycine, l-arginine, l-lysine, conjugated linoleic acid, phosphatidylserine/phosphatidylcholine complex,

[1] A copy of Stumvoll et al. is attached as Attachment A.

ornithine alpha-ketoglutarate, medium chain triglycerides and lecithin because all these agents are useful and effective for providing diet supplement and suitable for nutritional diet as taught by individual references" (Action at pages 8-9).

Gardiner teaches, however, the administration of three different supplements, *independently* of one another. Supplement 1 comprises: acetyl-l-carnitine, l-glutamine, l-leucine, ornithine alpha-ketoglutarate (OKG) whereas Supplement 3 comprises: creatine monohydrate, l-arginine, l-glycine. Gardiner does not teach that the components of the Supplements 1 and 3 can be combined. Rather, Gardiner explicitly teaches that Supplements 1 and 3 are administered *independently* (column 4, lines 16-20).

Moreover, many of the individual components that the examiner proposes to combine to arrive at the claimed invention are selected from compositions which are used for different purposes. For example, Gardiner's supplement is used to increase muscle size and strength for body builders and promote muscle adaptation to strenuous exercise, whereas Riley's supplement is used to assist in the reduction of risk factors of chronic disease such as coronary heart disease, cancer, and Syndrome X, and Michnowski's supplement is a snack that provides quick energy. One of ordinary skill in the art would hardly be motivated to select one or more components from a composition used to treat Syndrome X, for example, with one or more components selected from a composition used to increase muscle size and strength, or one or more components selected from a snack food. There simply is no motivation to make these selections.

Further, while the action asserts at page 9 that "each element merely would have performed in the nutritional supplement as it did separately," no purpose is attributed to a number of the components identified by the examiner. For example, Brantman discloses no purpose for including medium chain triglycerides in its composition, and thus it is unclear as to why one of ordinary skill would be motivated to haphazardly select medium chain triglycercides from a composition comprising a total of 25 different components (Example 1). Similarly, Riley discloses no purpose for including alpha lipoic acid and grape seed extract in its composition and thus it is unclear as to why one of ordinary skill would be motivated to haphazardly select alpha lipoic acid and grape seed extract from a composition comprising a total of up

to 33 components particularly when the disclosure describes these components as being optional (*see* Table bridging columns 20 and 21, in which alpha lipoic acid and grape seed extract are present in a range from about 0.0 to about...).

Furthermore, the Product alert document discloses that phosphatidylserine/phosphatidylcholine complex improves mental alertness, but none of the other references provide any indication that improving mental alertness is desirable in the context of their objectives, and thus it is unclear as to why the skilled artisan would be motivated to combine the phosphatidylserine/phosphatidylcholine complex with components selected from a composition designed to increase muscle size and strength, components selected from a snack, etc.

In view of the foregoing, the applicants respectfully submit that the examiner is improperly performing hindsight reconstruction using the Applicant's disclosure as a template. The rejections should be removed.

RENEWED REQUEST FOR INTERFERENCE

The Applicants again reiterate their request for the declaration of an interference between this patent and Gardiner, '339.

CONCLUSION

Should the examiner wish to discuss the foregoing, or any matter of form or procedure in an effort to advance this application to allowance, she is respectfully invited to contact the undersigned attorney at the indicated telephone number.

Respectfully submitted,

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PERSPECTIVES IN BASIC SCIENCE

Role of glutamine in human carbohydrate metabolism in kidney and other tissues

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Role of glutamine in human carbohydrate metabolism in kidney and other tissues. Glutamine is the most abundant amino acid in the human body and is involved in more metabolic processes than any other amino acid. Until recently, the understanding of many aspects of glutamine metabolism was based on animal and *in vitro* data. However, recent studies using isotopic and balance techniques have greatly advanced the understanding of glutamine metabolism in humans and its role in glucose metabolism in the kidney and other tissues. There is now evidence that in postabsorptive humans, glutamine is an important glucose precursor and makes a significant contribution to the addition of new carbon to the glucose carbon pool. The importance of alanine for gluconeogenesis, viewed in terms of the addition of new carbons, is less than previously assumed. It appears that glutamine is predominantly a renal gluconeogenic substrate, whereas alanine gluconeogenesis is essentially confined to the liver. As shown recently, renal gluconeogenesis contributes 20 to 25% to whole-body glucose production. Moreover, glutamine has been shown not only to stimulate net muscle glycogen storage but also to stimulate gluconeogenesis in normal humans. Finally, in humans with type II diabetes, conversion of glutamine to glucose is increased (more so than that of alanine). The available evidence on the hormonal regulation of glutamine gluconeogenesis in kidney and liver and its alterations under pathological conditions are discussed.

Glutamine is unique among the body's amino acids in many respects. It is the most abundant amino acid in blood and in the intracellular free amino acid pool of most tissues [1–5]. Moreover, glutamine is involved in more metabolic processes than any other amino acid (Table 1). Finally, its extensive utilization as a fuel by various organ systems and the marked reduction in its plasma concentration during critical illness have led to the proposal that it should be considered a conditionally essential amino acid [6].

Key words: gluconeogenesis, glycogenolysis, amino acid, carbon transport, metabolic pathways, hypoglycemia.

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During the past 15 years, glutamine has been the subject of several reviews addressing many of these issues [7–14]. However, most of these have been based on animal data, and none have dealt in any great detail with the role of glutamine in carbohydrate metabolism. There are considerable interspecies differences regarding circulating concentrations, net balance, and interorgan fluxes of glutamine. Consequently, data from animal experiments cannot be assumed to be applicable to humans [11].

Recently, the development of methods that permit the accurate determination of glutamine specific activity and enrichment in plasma [15–17] has facilitated the study of glutamine metabolism in humans. New insight has been gained on the human physiology of glutamine and its role as substrate and regulator for such processes as gluconeogenesis, glycogen formation, and protein synthesis/degradation. Glutamine has long been known to be a major substrate for renal gluconeogenesis, which has recently been re-evaluated by isotope studies in humans. This review summarizes new knowledge regarding the significance of glutamine for human carbohydrate metabolism and compares certain aspects of glutamine physiology with those of alanine, which has long been considered the pre-eminent gluconeogenic amino acid and the major vehicle for interorgan carbon transport.

BIOCHEMISTRY AND PHYSIOLOGY OF GLUTAMINE METABOLISM

Synthesis and degradation of glutamine

Glutamine can be synthesized by most tissues from α-ketoglutarate and glutamate via glutamate aminotransferase and glutamine synthetase, both cytosolic enzymes. Its major metabolic pathways are shown in Figure 1. Glutamine is formed by a reversible reaction involving the coupling between the exergonic cleavage of adenosine triphosphate (ATP) to adenosine diphosphate and inorganic phosphate and the endergonic addition of ammonia to glutamate. The carbon of α-ketoglutarate destined to become glutamine can come from glucose [18]

Table 1. Biological functions of glutamine

Interorgan nitrogen transport and intracellular nitrogen donor (renal ammoniagenesis, hepatic ureagenesis, synthetic processes)
Oxidative fuel (enterocytes, cells of the immune system)
Substrate and stimulator of glycogen synthesis
Substrate and stimulator of gluconeogenesis
Maintenance of acid-base balance
Potential regulator function of protein synthesis of unknown mechanism
Precursor for γ-aminobutyric acid in brain
Inhibitor of lipolysis and ketogenesis in animals
Relevance for total parenteral nutrition

and from other amino acids liberated from protein [19–21]. Free glutamine may also be formed as a direct result of proteolysis.

The initial step in glutamine degradation involves its conversion to glutamate by the action of phosphate-dependent glutaminase, an enzyme located in the mitochondrial matrix, and is closely linked to the glutamine transport system into the mitochondrial matrix [8]. The resultant glutamate can be either transaminated via glutamate-oxaloacetate-transaminase or glutamate-alanine-transaminase (cytosol and mitochondrial matrix) or, less importantly, can be deaminated via glutamate dehydrogenase (mitochondrial matrix). Both reactions yield α-ketoglutarate, an intermediate of the tricarboxylic acid cycle. Transport of glutamate into the mitochondrium involves either the electroneutral glutamate/hydroxyl antiporter system or the electrogenic glutamate/aspartate antiporter [8]. In some tissues (for example, jejunum), glutamate can also be converted to glutamate semialdehyde (glutamate semialdehyde dehydrogenase), with subsequent formation of proline and arginine. In most tissues, the major fate of glutamine is conversion to CO_2; however, in tissues possessing the appropriate enzymatic machinery, it can be converted to glucose (that is, liver and kidney; discussed later here) and glycogen (that is, liver and muscle).

Gluconeogenic and glycogenic pathway

The biochemical pathway for glutamine conversion to glucose in hepatocytes and renal tubular cells involves deamination to glutamate, transamination to α-ketoglutarate and conversion to oxaloacetate, a Krebs cycle intermediate, that enters the common gluconeogenic pathway (Fig. 1). Either carbons 1 through 3 or 2 through 4 of glutamine are directly incorporated into glucose, whereas the other carbons are lost as CO_2 in the Krebs cycle, and the phosphoenolpyruvate carboxykinase step can, in theory, re-enter the gluconeogenic pathway in the pyruvate carboxylase step. Unlike gluconeogenesis from other substrates (lactate, alanine, pyruvate), glutamine gluconeogenesis is unique in that it represents an exergonic reaction with a net yield of 8 mol ATP per mol glucose synthesized, provided there is aerobic oxidation of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH) [22–24]. This is due to the fact that reducing equivalents are generated in the glutamate dehydrogenase, as well as in the α-ketoglutarate dehydrogenase, malate dehydrogenase, and succinate dehydrogenase reactions in the Krebs cycle, steps not involved in the gluconeogenesis from other substrates.

Glutamine can not be directly incorporated into glycogen. Rather, its carbons enter the glucose-6-phosphate pool via gluconeogenesis, which is subsequently mutated to glucose-1-phosphatase. After activation with uridine-triphosphate and the formation of uridine diphosphate (UDP)-glucose, the carbon skeleton is incorporated into glycogen [25].

Interorgan glutamine metabolism

In humans, glutamine accounts for approximately 20% of all amino acids in the circulation, almost twice as much as alanine, which is widely considered to be the most important gluconeogenic amino acid [26–31]. The basal plasma turnover rate of glutamine in postabsorptive normal subjects (approximately 5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) is slightly greater than that of alanine (approximately 4.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), and the plasma concentration of glutamine (approximately 0.6 mM) is about twice that of alanine (approximately 0.25 mM) [32–40].

The concentration of glutamine in plasma is determined by its relative rates of release into and uptake from plasma by various tissues. The major tissues releasing net amounts of glutamine into plasma are skeletal muscle, lungs, and adipose tissue. Gut and kidney are the main organs demonstrating net uptake of glutamine. Liver and muscle play major regulatory roles in glutamine homeostasis because, depending on the particular circumstances, both can markedly alter either their release or uptake of glutamine (Table 2), but these have generally not been quantitated to any great extent in humans (Fig. 2).

GLUTAMINE AS GLUCONEOGENIC SUBSTRATE

General considerations, hepatic and renal gluconeogenesis

Gluconeogenesis, synthesis of glucose from nonglucose precursors [41, 42], accounts for approximately 50% of all glucose released into the circulation after an overnight fast in humans [43]. It becomes crucial with more prolonged fasting for the maintenance of the glucose supply for the nervous system and anaerobic energy production when glycogen stores become depleted [41, 44]. In humans, lactate, glycerol, and amino acids account for nearly all of the carbons transported through the circulation and incorporated into plasma glucose [24, 42]. In the postabsorptive state, more plasma glucose is converted to plasma lactate than comes from plasma

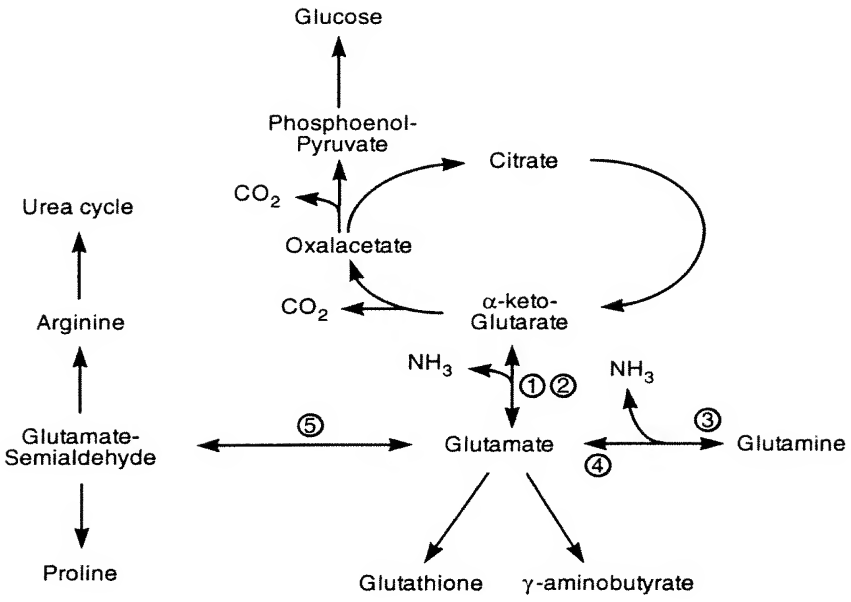

Fig. 1. Biochemistry of glutamine metabolism (main pathways and key enzymes): (*1*) glutamate dehydrogenase, (*2*) glutamate oxaloacetate transaminase, (*3*) glutamine synthetase, (*4*) glutaminase, (*5*) glutamate semialdehyde dehydrogenase.

Table 2. Tissue glutamine net uptake and release in postabsorptive humans

Tissues	Net uptake	Net release	Reference
	μmol/min		
Muscle	—	59 (19–168)	[28, 30, 31, 33, 111, 200–211]
Splanchnic tissues	97 (59–165)	—	[77, 111, 187, 188, 209, 211, 214]
Gut	57 (41–78)	—	[94, 111, 188, 215]
Liver	20	—	[111]
Kidney	60 (35–110)	—	[55, 62, 63, 209, 214, 216]
Brain	13 (5–23]	—	[209, 214, 216]
Lung	—	56	[217]
Adipose tissue	—	12	[218]

lactate [45, 46], so that lactate gluconeogenesis actually provides no net addition of carbon to the glucose pool. Glycerol becomes a quantitatively important gluconeogenic precursor only when there is accelerated lipolysis such as after prolonged fasting [47] and in diabetes mellitus [48]. Consequently, under normal circumstances, amino acids are largely responsible for the net addition of carbons to the glucose pool, which are not immediately derived from plasma glucose.

Gluconeogenesis is essentially limited to liver and kidney because other tissues lack glucose-6-phosphatase. Both organs are comparably equipped with a complete set of gluconeogenic enzymes [44]. Although it has long been recognized that on a gram-for-gram tissue basis, the gluconeogenic capacity of the kidney exceeds that of the liver [49], until recently, the human kidney has generally been regarded as contributing insignificantly to postabsorptive glucose production [50–52]. This traditional view was based on net balance data of glucose, that is, the mathematical product of arteriorenal venous difference of glucose concentrations (obtained through a sampling catheter in a renal vein) and renal blood flow showing no significant difference in arterial and renal vein glucose concentrations in the basal state [53–55]. Thus, by merely representing the difference between uptake and release of a substrate, net balance measurements cannot evaluate the contribution of an organ to the entry and removal of a substrate from the systemic circulation.

Moreover, inferences based on net balance measurements may lead to an underestimation of the role of an organ in the overall metabolism of a substrate. For example, with isotope dilution determination of systemic glucose flux, entry of glucose into the circulation is quantitated by the dilution of the plasma glucose tracer concentration by unlabeled glucose released into the circulation [56]. If the kidney were to take up and release glucose at equal rates, there would be no arteriorenal venous glucose difference, and net glucose balance would be zero. Nevertheless, release of unlabeled glucose into the circulation by the kidney would dilute the plasma glucose tracer concentration and would contribute to the isotopic estimation of glucose entry into the circulation (Fig. 2). Similar considerations hold for the contribution of the kidney to removal of glucose from the circulation as determined isotopically. Therefore, a combination of net balance and isotopic techniques with

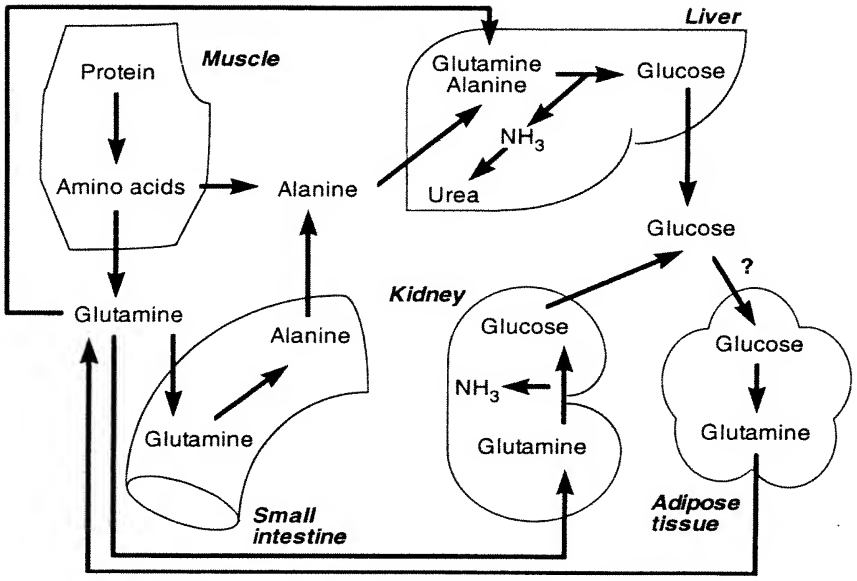

Fig. 2. Release and uptake of glutamine by various organs in humans. Approximations are based on net balance and, where available, on isotope data.

measurement of substrate as well as tracer concentrations is necessary to assess individually the uptake and release of a substrate by an organ [46, 56, 57].

In 1978, Kida et al found a renal contribution of 25% to systemic glucose production in rats by injecting a bolus of [^{14}C]glucose and by comparing the decline of blood glucose concentration and [^{14}C]glucose concentration following hepatectomy [58]. In intact animals, the isotopic net balance approach was not employed until recently, when a significant renal uptake of glucose was shown in dogs, accounting for as much as 30% of glucose removal from the circulation under postabsorptive conditions [59, 60]. Because renal net balance was within the expected range, that is, close to zero, the data also indicated that the kidney was responsible for more than 20% of glucose entry into plasma in postabsorptive dogs.

An analogous approach was recently used in healthy, postabsorptive humans [61]. These studies showed, as expected, that the human kidney simultaneously takes up and releases appreciable amounts of glucose. Renal glucose release accounted for approximately 25% of all glucose released into the circulation, and its uptake of glucose accounted for approximately 20% of all glucose removed from the circulation (Table 2) [61–63]. These results thus refuted textbook wisdom that stated that human kidneys play a minor role in glucose homeostasis.

Because the normal human kidney contains negligible amounts of glycogen [64] and cells other than proximal tubules that could theoretically store glucose lack glucose-6-phosphatase [65, 66], gluconeogenesis is likely responsible for essentially all renal glucose release. In postabsorptive humans, renal glucose release accounts for 15 to 25% of all glucose released into the circulation, and gluconeogenesis accounts for approximately 40 to 50% of systemic glucose release [43]. It can therefore be readily calculated that under these circumstances, the human kidney should account for approximately half of all gluconeogenesis and should thus be as important as a gluconeogenic organ as the liver [67]. Available evidence for glutamine gluconeogenesis in both organs is reviewed.

Early *in vitro* and animal studies

In 1963, glutamine was first shown to be an efficient substrate *in vitro* for renal and subsequently also for hepatic gluconeogenesis [68, 69]. The first demonstration of the gluconeogenic potential of the glutamate/glutamine couplet in mammals *in vivo* dates back to 1968 [70]. As much as 30 to 40% of label injected intraportally as [^{14}C]glutamate to lactating cows was incorporated into plasma glucose. Based on specific activities in plasma glucose, the authors conservatively estimated that approximately 8% of glucose carbons were derived from glutamate. Intraportal injection of various ^{14}C-labeled precursors in rats also demonstrated glutamine incorporation into glucose, albeit at a lower rate than for alanine and lactate [71]. Tracer data in sheep have shown that 40 to 60% of all amino acids incorporated into glucose are accounted for by alanine plus the glutamate/glutamine couplet [72–74], and approximately 5% of plasma glucose comes from plasma glutamine [75]. It was estimated that in sheep, the glutamine/glutamate couplet could be responsible for 20 to 40% of renal glucose release and approximately 20% of overall glucose release [73]. However, considerable species differences for rumi-

nants have to be taken into consideration [75, 76]. Most of our knowledge regarding the regulation of glutamine gluconeogenesis is still based on *in vitro* experiments and studies in animals (discussed in the **Regulation of glutamine gluconeogenesis** section).

Human studies

In 1971, Marliss et al were the first to demonstrate net splanchnic uptake of glutamine in humans and proposed that glutamine was an important gluconeogenic precursor [77]. The relative contributions of liver and gut to splanchnic glutamine uptake, however, were not assessed in this study. It is remarkable that although some of the early glutamine studies from the late 1960s and 1970s were done in Krebs' laboratory [22, 68, 69, 78], in his 1980 review, he described glutamine as the most versatile amino acid but made no mention of it as a gluconeogenic precursor [79]. A probable explanation is that the liver was considered to be the predominant gluconeogenic organ at that time, and human studies in the late 1960s and 1970s had indicated that most, if not all, net splanchnic glutamine uptake was due to nonhepatic tissues, whereas hepatic uptake of alanine exceeded that of all other amino acids measured [80, 81]. Therefore, the contribution of glutamine to hepatic gluconeogenesis was assumed to be of minor importance, and it became widely held that alanine was the pre-eminent gluconeogenic amino acid [80, 82, 83]. In none of these studies, however, was glutamine incorporation into glucose actually quantitated and compared with that of alanine.

The recent development of a method to determine the specific activity and enrichment of [^{14}C]glutamine and [^{13}C]glutamine, respectively, in plasma [15–17] made it feasible to trace the incorporation of glutamine carbons into plasma glucose in humans. The use of a combination of isotopic tracers showed that in normal postabsorptive humans, incorporation of plasma glutamine into plasma glucose accounted for approximately 5 to 8% of overall glucose production [33, 34, 40]. A large proportion of plasma glutamine is oxidized to CO_2. Some of this CO_2 could, in theory, be incorporated into glucose through simple fixation by pyruvate carboxylase and not represent true gluconeogenesis [84]. However, using incorporation into glucose of carbon derived from infused carbon-labeled leucine as an index for CO_2 fixation, it has recently been demonstrated that only 4% of plasma glutamine conversion to plasma glucose is due to simple fixation of CO_2 [40].

Rates of glutamine gluconeogenesis in humans obtained from ratios of [^{14}C]glucose- to [^{14}C]glutamine-specific activities in plasma [33, 34] represent whole-body glutamine incorporation into glucose and cannot assess individual contributions by liver and kidney. In humans, net hepatic glutamine uptake (approximately 30 μmol/min) is relatively small compared with that of alanine (105 μmol/min) [80, 85] and cannot account for the isotopically determined whole-body glutamine conversion to glucose (approximately 45 μmol/min) [33, 34]. Indeed, the latter represents only a minimal estimate due to dilution of specific activity by carbon exchange in the Krebs cycle [86–88]. Furthermore, it is likely that some glutamine taken up by the liver serves as a shuttle for nitrogen transport, protein synthesis, and other metabolic purposes and does not undergo gluconeogenesis. Therefore, based on net hepatic uptake, it appears that only a minor proportion of glutamine gluconeogenesis occurs in the liver.

In healthy postabsorptive volunteers, the rate of conversion of glutamine to glucose by the kidney was directly assessed using a combination of isotopic ([U-^{14}C]glutamine, [6-^{3}H]glucose) and renal vein catheterization techniques, which allows the calculation of hepatic glutamine gluconeogenesis as the difference between systemic and renal. It was found that almost 80% of systemic glutamine gluconeogenesis took place in the kidney and that 10 to 20% of renal glucose production was accounted for by renal glutamine gluconeogenesis [62, 63]. This can explain the failure of hepatic glutamine uptake to account for all of the glutamine gluconeogenesis in humans. Furthermore, the findings identify glutamine as a major renal gluconeogenic precursor in humans and confirm numerous earlier reports from *in vitro* studies using liver tissue or isolated hepatocytes [68, 89–91] and renal cortex slices [22, 69, 78, 92].

Comparison of alanine and glutamine as gluconeogenic substrates

In animals [68, 72, 73, 75, 93] and humans [33, 34], alanine and glutamine are the two predominant gluconeogenic amino acids, accounting for 40 to 70% of all amino acids converted to glucose. Differences in physiologic roles and metabolic origins justify comparative assessment of these two amino acids and their respective contributions to gluconeogenesis in humans.

Carbon transport through plasma. It is well established that alanine carbon can, among other sources, originate from glucose carbon in a variety of tissues [94–99]. Alanine derived from glucose as well as from other precursors can then be released into the circulation and utilized for production of glucose [33, 46, 100, 101]. This shuttle of carbon between plasma glucose and alanine is commonly referred to as the glucose-alanine cycle [83, 102], analogous to the Cori cycle involving plasma glucose-lactate interconversions [103, 104]. Until recently, it was not known whether a similar cycle between glucose and glutamine existed in humans.

As assessed by incorporation of carbon-labeled glucose into alanine and glutamine, 40% of plasma alanine, but only about 15% of plasma glutamine, came from plasma glucose [18]. Approximately 25% of plasma ala-

Table 3. Gluconeogenic substrates in postabsorptive healthy humans

Gluconeogenic substrate	Gluconeogenesis *μmol/kg/min*	Substrate recycled from glucose %	"True" gluconeogenesis *μmol/kg/min*	Glucose from new carbons added by substrate %
Lactate	2.40	67	0.79	32
Glutamine	0.85	13	0.74	30
Alanine	0.80	40	0.48	20
Glycerol	0.20	0	0.20	8
Other amino acids	0.27	10	0.24	10
Sum	4.42	100	2.45	100

Assumptions are: systemic glucose production = 11.3 μmol/kg/min, gluconeogenesis = 40% of systemic glucose production, other amino acids contribute approximately 5% to total gluconeogenesis, all other amino acid not glucose derived, Krebs-cycle carbon exchange factor 1.4. Data are taken from [18,33,40,48,87,193].

nine and 10% of plasma glutamine were converted to plasma glucose in humans [33, 34, 62, 105]. Furthermore, approximately 30% of plasma alanine and 45% of plasma glutamine came from the direct release from protein [33]. These findings provide evidence that in postabsorptive humans, there is greater flux of carbon from plasma glucose to plasma alanine than from plasma alanine to plasma glucose. The opposite is true for glutamine, because a large proportion of plasma glutamine originates from glutamine in protein or from other amino acids released from protein.

Knowledge of the relative flux of glucose-derived carbon to alanine and glutamine, respectively, versus the flux of non-glucose-derived amino acid carbon ("new carbon") to glucose challenges the widely held view that alanine is the most important gluconeogenic amino acid [80, 82, 83]. Table 3 shows the rates of gluconeogenesis from the major gluconeogenic precursors. Because a certain proportion of each precursor comes from plasma glucose, rates of gluconeogenesis to a certain extent reflect mere recycling of carbons back to glucose. True gluconeogenesis or "neo-gluconeogenesis" [106], that is, glucose synthesis from nonglucose-derived precursors, is obtained by subtracting the recycled proportion from total gluconeogenesis. Thus, in terms of adding new, that is, nonglucose-derived, carbons to the plasma glucose pool glutamine appears to be the most important amino acid and is as important as lactate.

Organ selectivity. In vitro experiments [24] and net balance studies in humans [77, 80, 81] have suggested that alanine and glutamine are selective gluconeogenic precursors for liver and kidney, respectively. Recent studies in healthy postabsorptive volunteers using a combination of isotopic ([^{14}C]glutamine and [^{13}C]alanine) and renal vein catheter techniques compared systemic and renal rates of conversion to glucose of these amino acids. Although almost 80% of systemic glutamine gluconeogenesis took place in the kidney, alanine gluconeogenesis was virtually absent in the kidney and, therefore, must have occurred exclusively in the liver [62].

These results are consistent with the observations of Björkman and Felig, who found that infusion of alanine into 60-hour fasted normal volunteers increased net splanchnic glucose release but had no effect on net renal glucose release [54]. The finding in postabsorptive humans that hepatic gluconeogenesis from alanine is approximately fivefold greater than that from glutamine [62] is consistent with *in vitro* studies indicating that glucose production from alanine by perfused rat livers and isolated rat hepatocytes considerably exceeds that from glutamine [68, 71, 90, 107]. There are, however, species differences in hepatic gluconeogenesis [108], and most *in vitro* studies have used supraphysiological substrate concentrations (for example, 10 mM) [68, 108]. For example, in rat kidney, glucose production from glutamine is maximal at 2 mM glutamine, whereas glucose production from lactate is maximal at 5 mM lactate; however, at 1 mM glutamine and lactate, glucose production from glutamine exceeds that from lactate [109].

Because renal uptake of alanine was approximately half that of glutamine while its conversion to glucose was less than one tenth that of glutamine [62], differences in uptake or transport of alanine and glutamine probably cannot explain the selectivity in the use of these amino acids for gluconeogenesis by the kidney. Activity of enzymes of alanine metabolism, however, is much lower in the kidney than in the liver [68, 78]. Proximal cortical tubules, where gluconeogenesis primarily takes place in the human kidney, are known to lack or have very little alanine aminotransferase activity [110]. This would severely limit conversion of alanine to pyruvate, an essential step in the formation of glucose from alanine, and would explain the limited gluconeogenic use of alanine by the human kidney.

There are several possible explanations for the difference in renal and hepatic use of glutamine for gluconeogenesis. First, in terms of substrate supply, concentrations of glutamine presented to the kidney are likely to be greater than those presented to the liver. Portal venous glutamine levels are lower than arterial levels [111–113] because of intestinal glutamine extraction [114], and portal venous blood flow is approximately three times greater than hepatic arterial flow [112]. Second, glutamine is transported across the plasma membrane in liver

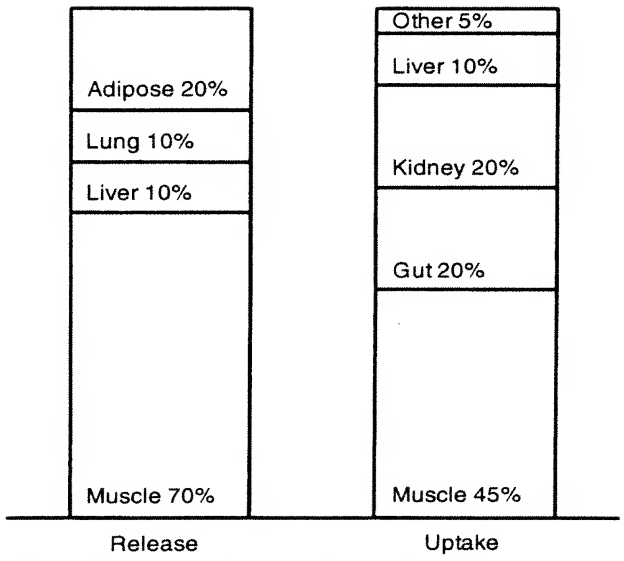

Fig. 3. Interorgan glutamine metabolism (principal fluxes).

specifically by the N system, whereas in kidney, it is mainly transported by the A system [115]. There are several differences in these transport systems (for example, the A system, but not the N system, is stimulated by hormones such as glucagon and epinephrine) [115, 116]. Third, although transport across the hepatocyte plasma membrane appears to be the rate-limiting step for metabolism of alanine [117, 118], there is evidence that glutaminase is the rate-limiting step for glutamine metabolism [8]. Liver and kidney glutaminase differ in many respects [119], one of which being a relatively low glutaminase activity in liver [120]. Thus, a combination of differences in substrate supply, transport, and enzymatic activity of rate-limiting steps might explain differences in the use of glutamine by the liver and kidney for gluconeogenesis.

The exclusive use of alanine for gluconeogenesis by the liver in humans suggests that incorporation of carbon-labeled alanine into glucose may serve as a noninvasive metabolic probe for investigation of hepatic gluconeogenesis (Fig. 3) [62].

Regulation of glutamine gluconeogenesis

One might expect that factors regulating gluconeogenesis in general [121–124] should affect gluconeogenesis from glutamine in a similar fashion. However, because glutamine incorporation into glucose occurs predominantly in the kidney, in some aspects, its gluconeogenesis is unique.

Hormones

Insulin. In humans during euglycemic-hyperinsulinemic clamp experiments, systemic glucose production, which represents the sum of hepatic plus renal, decreases virtually to zero [125]. This strongly suggests that insulin suppresses renal and hepatic gluconeogenesis from all substrates in humans. Infusion of insulin in normal volunteers, which increased arterial insulin levels from 36 to 219 pM suppressed systemic glutamine gluconeogenesis by 50%. The fact that glutamine gluconeogenesis in the liver was reduced by approximately 25%, whereas that in kidney was reduced by almost 75%, suggests that renal glutamine gluconeogenesis is more sensitive to insulin than hepatic [126]. Renal glutamine uptake was not influenced by insulin in this study. Many studies have shown that insulin inhibits gluconeogenesis *in vitro* in kidney cortex slices [127] and *in vivo* in diabetic animals treated with insulin [58]. In normal dogs, intrarenal infusion of insulin, designed not to suppress systemic glucose appearance, decreased renal glucose production of the infused kidney by approximately 75% [60]. In sheep, insulin had no effect on hepatic glutamine net balance [128]. In dogs, insulin suppressed renal glucose production by 75% and renal glycerol incorporation into glucose by 30%. Because glutamine is a major renal gluconeogenic precursor, insulin should also suppress renal gluconeogenesis from glutamine.

Glucagon. In humans, infusion of glucagon designed to increase plasma glucagon concentrations to those observed during hypoglycemia increased overall glutamine gluconeogenesis by 25%, and this increase was entirely accounted for by increased hepatic glutamine gluconeogenesis [63]. The fact that this stimulation occurred in the absence of significant changes in plasma glutamine concentration or turnover suggests increased gluconeogenic efficiency. In sheep, infusion of glucagon decreased plasma glutamine concentration by 30% and doubled net hepatic glutamine uptake [128, 129]. The failure of glucagon to stimulate renal glutamine gluconeogenesis in humans is in agreement with *in vitro* studies demonstrating that glucagon does not stimulate renal gluconeogenesis [130]. Moreover, those segments of the nephron equipped with gluconeogenic enzymes (proximal tubule) [65, 66] lack glucagon receptors that have nevertheless been identified on cells of the distal tubule [131]. Preliminary data indicate that infusion of glucagon in humans, while maintaining insulin and glucose concentrations constant (somatostatin clamp), reduces plasma glutamine by 30% but does not affect overall release into the circulation [132]. This increased plasma clearance could have represented increased hepatic uptake and utilization for gluconeogenesis provided that there were reciprocal changes in uptake in other tissues.

In isolated hepatocytes from starved rats, glucagon in-

creased gluconeogenesis from glutamine to a far greater extent than that from any other amino acid precursor, including alanine, serine, and proline [91]. The authors suggested that provision of ATP in the mitochondria may be responsible, as it has been shown that addition of glucagon to cells metabolizing glutamine results in an increase in the intramitochondrial ATP/adenine diphosphate ratio [133]. In addition, glucagon activation of mitochondrial glutaminase has been proposed to play a role, but the exact mechanism is still unclear.

Catecholamines. In a recent study in healthy humans that was designed to measure renal glucose production isotopically, epinephrine acutely increased systemic glucose production by 60%, hepatic glucose release by 50%, and renal glucose production by 100% [61]. It is of note that in this study, infusion of epinephrine, which resulted in circulating concentrations of epinephrine similar to those observed during hypoglycemia [134], caused a sustained increase in renal glucose release that by three hours accounted for essentially all of the increased appearance of glucose in the circulation. The stimulatory effect of epinephrine on renal glucose production could be direct via cAMP-mediated stimulation of renal key gluconeogenic enzymes, as shown *in vitro* [135], or indirect through increased substrate availability because epinephrine has been shown to increase the availability of gluconeogenic precursors [136].

Infusion of epinephrine designed to increase plasma epinephrine concentrations to levels observed during severe hypoglycemia increased renal glutamine uptake nearly 80% in humans [62]. In this study, renal glucose release increased approximately twofold. Overall glutamine gluconeogenesis was stimulated by 75%, and the proportion accounted for by the kidney increased from 73 to 90%, whereas the hepatic contribution decreased from 27 to 10%.

During insulin-induced hypoglycemia in healthy volunteers, renal glucose release measured isotopically was increased almost threefold compared with the euglycemic control experiment [137, 138]. Thus, renal glucose production is stimulated by counterregulatory hormones, primarily catecholamines, and plays an important role in glucose counterregulation of hypoglycemia.

Other hormones. In vitro studies have shown that glucocorticoids, among other hormones (thyroxine, growth hormone, parathyroid hormone), increase renal glucose release and in some instances gluconeogenesis, although that of glutamine has not been specifically examined [67, 139]. In sheep, the glucocorticoid dexamethasone only marginally increased overall glutamine gluconeogenesis, but the proportion that was accounted for by the kidney increased disproportionately [75]. In humans, infusion of hydrocortisone increased plasma glutamine flux by 30% [140]; however, gluconeogenesis was not assessed in this study.

Nonhormonal factors

Among the many other factors influencing gluconeogenesis in general such as nutritional state diet, acid-base status, and hypoglycemia [121, 122, 124], only few data are available regarding glutamine gluconeogenesis. For in-depth coverage of *in vitro* and animal data on regulation of renal gluconeogenesis, the reader is referred to two extensive review articles [24, 139].

There is limited data on fasting in humans. In one study, glutamine gluconeogenesis increased by 43% during prolongation of a fast from 18 to 42 hours, whereas its contribution to total gluconeogenesis remained unchanged [40]. In humans, net renal glucose release has been correlated with the severity of acidosis [141], but no specific data are available for glutamine gluconeogenesis. Pitts et al reported that during chronic acidosis, as much as 40% of the glutamine extracted by the kidney could have been converted to glucose [142]. Because of renal Krebs cycle carbon exchange, however, this probably represents an underestimation [22]. In 72-hour experimentally acidotic sheep, systemic glutamine gluconeogenesis was unchanged [75]. In this study, however, individual contributions of liver and kidney were not assessed. Because renal glutamine uptake increased during acidosis in sheep [75], it is possible that renal glutamine gluconeogenesis increased, whereas hepatic glutamine gluconeogenesis decreased. *In vitro* studies have demonstrated that acidosis increases renal production of glucose from glutamine [143–145] or glutamate [146]; this could be explained by increases in the activities of glutaminase, glutamate dehydrogenase, and phosphoenolpyruvate carboxykinase demonstrated to occur during acidosis [147].

The study of acidosis is of particular interest because glutamine is the most important substrate for renal ammoniagenesis [22, 143, 145, 146]. During acidosis, animal kidneys extract increased amounts of glutamine (five times more than the gut) for generation of ammonia and bicarbonate [148]. The dog liver switches from net uptake to net release [149–151], and net muscle glutamine release increases in various species [11, 148, 152, 153]. These extrarenal adaptations would make glutamine available for the kidney to provide carbon and nitrogen essential for base generation at the expense of hepatic ureagenesis [11, 148, 154, 155]. Increased renal glucose formation is currently viewed as an outlet for the increased formation of α-ketoglutarate derived from glutamate and glutamine, thus conserving carbon (Fig. 4).

Methodological considerations in renal net balance and isotopic studies

The use of a combination of balance and isotopic techniques in some of the reviewed studies in humans has certain shortcomings that need to be taken into consider-

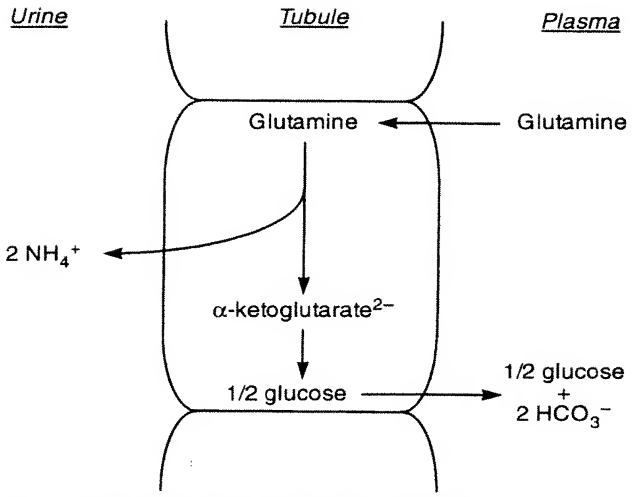

Fig. 4. Renal ammoniagenesis and gluconeogenesis.

ation. First, the calculation of renal production of glucose from glutamine and alanine depends on several determinations (for example, renal blood flow, substrate concentrations, substrate-specific activities, and enrichments), each of which involves some measurement error. Imprecision in each of these, although small, will lead to larger imprecision in the final calculation. The errors, however, are not strictly additive. For example, in studies from our own laboratory [62, 63], coefficients of variation (CV) of blood flow, concentrations, and specific activities were less than 2%, 3%, and 5%, respectively. The overall CV is equal to the square root of the sum of the individual CVs or approximately 6%. Second, isotopic assessment of gluconeogenesis using a labeled precursor will underestimate the incorporation of that precursor into glucose because of dilution of the specific activity or enrichment of the labeled precursor in the Krebs cycle and other pools [22, 87, 88, 156, 157]. Underestimation because of Krebs cycle carbon exchange that occurs in both liver and kidney [22, 88] will depend on the location of the label in the precursor used and on the specific experimental conditions [88, 108]. In postabsorptive humans, this underestimation has been calculated to be as great as 40% [87].

Krebs cycle carbon exchange has not been directly compared; however, *in vitro* data for glutamine incorporation into glucose by canine renal tubules [22], and *in vivo* data for incorporation of lactate into glucose in dogs [156] indicate comparable degrees of underestimation. Therefore, it is reasonable for the previously mentioned studies in humans to assume that hepatic and renal carbon exchange are similar. Isotopic dilution would thus affect the absolute rates of incorporation into glucose but not the relative gluconeogenesis in liver and kidney.

GLUTAMINE AS A REGULATOR IN CARBOHYDRATE METABOLISM

In addition to being a metabolic substrate, glutamine may also act as a regulator of several physiologic processes. For example, *in vitro* glutamine has been shown to stimulate lipid formation by activation of acetyl-CoA carboxylase [158] and to inhibit adipocyte lipolysis [159]. Moreover, glutamine has been reported to suppress proteolysis in liver and skeletal muscle [160–163] and to stimulate protein synthesis in skeletal muscle [160, 164]. In humans, glutamine infusion has been shown to suppress its own *de novo* synthesis by almost 50% [165, 166]. Finally, as will be discussed, glutamine has been shown to stimulate hepatic and renal gluconeogenesis as well as glycogen synthesis in muscle and liver.

Gluconeogenesis

Studies employing isolated renal tubules have demonstrated that glutamine stimulates incorporation of fructose, dihydroxyacetate, and lactate into glucose [109]. In normal postabsorptive volunteers infused with glutamine at a rate estimated to approximate its appearance in plasma after a protein meal, a combination of isotopic and forearm balance techniques was used to assess changes in glutamine gluconeogenesis and forearm substrate metabolism [165]. Neither systemic glucose turnover nor forearm balance of glucose and alanine were altered. Although infusion of glutamine increased plasma glutamine concentration and turnover only threefold, formation of glucose from glutamine increased sevenfold. Formation of glucose from alanine was also stimulated in the absence of a change in plasma alanine concentration. Because glutamine and alanine appear to be selective substrates for renal and hepatic gluconeogenesis, respectively [62], these observations imply that infusion of glutamine had increased gluconeogenesis in both organs. Moreover, because the stimulatory effects of glutamine on gluconeogenesis occurred in the absence of changes in plasma insulin and glucagon levels, these results provide evidence that in humans, glutamine may act as both a substrate and a regulator of gluconeogenesis. Finally, because increases in the concentrations of other gluconeogenic precursors such as lactate [167], alanine [71, 168], and glycerol [48] produce only proportional increases of their incorporation into glucose, these observations provide evidence that glutamine exerts a unique stimulatory effect on gluconeogenesis.

There are several possible mechanisms by which glutamine may regulate gluconeogenesis. Glutamine could substitute as an oxidative fuel for other substrates and shunt them into the gluconeogenic pathway. In addition, it has been reported to increase lactate uptake by renal tubules [109] and to increase the activity of phosphoenolpyruvate carboxykinase in rat kidney cortex homoge-

nates [169]. Moreover, in cultured HeLa cells and fibroblasts, glutamine has been shown to reduce fructose-2,6-biphosphate levels [170]. Such an effect, if operative in human liver and kidney, would promote gluconeogenesis by activating fructose-1,6-biphosphatase [171]. Finally, increased glutamine utilization in liver and kidney could stimulate the aspartate-malate shuttle [172] while generating the increased reducing equivalents and ATP required for increased gluconeogenesis [107].

Glycogen synthesis

Liver. It has been known for some time from studies by Katz, Golden and Wals [173, 174] and Rognstad [175] that not only glucose but also glycogen is formed by isolated liver preparations in the presence of gluconeogenic substrates, and that glutamine is the most effective amino acid for enhancing glycogen formation. In addition to simply providing carbons for indirect pathway glycogen synthesis [176–178], the activation of key enzymes such as glycogen synthetase by glutamine appears to be involved [158, 175]. It has been proposed that stimulation of glycogen synthetase is related to signals caused by hepatocyte swelling after rapid uptake of glutamine [179]. Liver cell swelling has also been observed with other metabolites [179, 180], but glutamine seems to cause the most marked volume changes [181]. The exact mechanism of this stimulation, however, is still unclear. Furthermore, there are conflicting observations from studies in perfused livers whether glutamine was incorporated into glucose but not into glycogen [182, 183]. Available evidence suggests there may be differential regulation by individual amino acids in directing carbons in the glucose-6-phophate pool toward generation of either glucose or glycogen [173, 181].

Muscle. The stimulatory effect of glutamine on glycogen synthesis is not confined to the liver. Administration of large intraperitoneal doses of glutamine in rats resulted in a significant increase in skeletal muscle glycogen accumulation [184]. In humans whose muscle glycogen and glutamine stores were depleted by exercise, infusion of glutamine at double its normal plasma flux, but not infusion of alanine plus glycine, increased net muscle glycogen storage threefold compared with infusion of saline [185]. Because labeling of glycogen by infused [^{13}C]glucose was similar in the glutamine and saline group, it was concluded that glutamine had no effect on the fractional rate of blood glucose incorporation into glycogen. The authors proposed that stimulation of glycogen synthetase and diversion of glutamine carbon to glycogen were both involved.

DIABETES MELLITUS

Concentration [186–188], turnover [36], and net splanchnic extraction of glutamine [188] are normal in patients with type I diabetes mellitus. Normal plasma glutamine concentrations and turnover rates have also been found in type II diabetic subjects, but splanchnic extraction has not been assessed [34, 189]. Using a combination of isotopic and forearm balance techniques, increased glutamine conversion to glucose and to alanine but decreased oxidation and forearm release were found in type II diabetics compared with normal controls [34]. It was suggested that the reduction in glutamine oxidation could be due to increased plasma free fatty acids (FFA) and glucose levels usually associated with type II diabetes [190]. Free fatty acids and glucose, in view of the prevailing hyperglycemia, could have substituted for glutamine as an oxidative fuel in certain tissues making more available for gluconeogenesis. Octanoate and ketone bodies have been shown to reduce glutamine utilization by rat enterocytes [191], and glutamine uptake by the small intestine is reduced in streptozotocin diabetic rats [191, 192].

In type II diabetes, glucose formed from glutamine has been shown to exceed that formed from alanine (0.90 vs. 0.78 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) [34]. However, net glutamine uptake by the human liver is small relative to that of alanine [81, 85]. Values reported for net hepatic alanine uptake in type II diabetes [80] can account for alanine conversion to glucose [34, 193], whereas those reported for net hepatic glutamine uptake (30 $\mu mol \cdot min^{-1}$ in normal volunteers) [85] can not account for the glutamine conversion to glucose (95 $\mu mol \cdot min^{-1}$) [34]. Furthermore, net splanchnic glutamine extraction has been reported not to be altered in type I diabetes [188]. It thus appears difficult to postulate increased hepatic glutamine gluconeogenesis in type II diabetes. One possible explanation is that there is increased release or decreased uptake of glutamine by nonhepatic splanchnic tissues. In streptozotocin diabetic rats, uptake of glutamine by the small intestine is decreased [191, 192, 194]. Alternatively, it is possible that in type II diabetes renal glutamine gluconeogenesis is increased. Renal glutamine uptake is increased in streptozotocin diabetic rats [192]. Moreover, in diabetic animals, there is increased activity of renal gluconeogenic enzymes [195–197] and increased renal gluconeogenesis [58, 144, 198]. Finally, preliminary studies indicate that in patients with type I diabetes taken off regular insulin, renal glucose release is increased [199].

CONCLUSIONS

Until recently, the importance in humans of glutamine as a gluconeogenic precursor and a potential metabolic regulator has been underestimated. There is now evidence that even in postabsorptive humans, glutamine makes a significant contribution to the addition of new carbon, that is, noncarbohydrate derived, to the glucose carbon pool. The importance of alanine for gluconeogen-

esis, viewed in terms of addition of new carbons, is less than previously assumed. It appears that glutamine is predominantly a renal gluconeogenic substrate, whereas alanine gluconeogenesis is essentially confined to liver. Both renal glucose production and renal glutamine gluconeogenesis are under hormonal control and contribute significantly to overall glucose production. Moreover, glutamine has been shown not only to stimulate net muscle glycogen storage in humans, but also to stimulate gluconeogenesis in normal humans. In humans with type II diabetes, conversion of glutamine to glucose is increased (more so than that of alanine). Various aspects of glutamine metabolism have been described in animal models. Because of considerable differences among species these need to be investigated in humans, for example, nutrient and hormonal regulation (effects of fasting, growth hormone, cortisol, and insulin) and the interrelation of glutamine metabolism with those of other substrates (for example, fatty acids). In addition, because glutamine gluconeogenesis occurs predominantly in the kidney, further studies are particularly needed on renal gluconeogenesis. Finally, the roles of glutamine in the regulation of postprandial glycogen and protein metabolism and in the control of substrate fluxes during counterregulation of hypoglycemia need to be elucidated.

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REFERENCES

1. Vinnars E, Bergström J, Fürst P: Influence of postoperative state on intracellular free amino acids in human muscle tissue. *Ann Surg* 182:665–671, 1975
2. Möller P, Alvestrand A, Bergström J, Fürst P, Hellström K: Electrolytes and free amino acid in leg skeletal muscle of young and elderly women. *Gerontology* 29:1–8, 1983
3. Roth E, Zoch G, Schultz F: Amino acid concentrations in plasma and skeletal muscle of patients with acute hemorrhage necrotizing pancreatitis. *Clin Chem* 31:1305–1309, 1985
4. Askanazi J, Carpentier YA, Michelsen CB, Elwyn DH, Fürst P, Kantrowitz LR, Gump FE, Kinney JM: Muscle and plasma amino acids following injury. *Ann Surg* 192:78–85, 1980
5. MacLean DA, Graham TE, Saltin B: Branched-chain amino acids augment ammonia metabolism while attenuating protein breakdown during exercise. *Am J Physiol* 267:E1010–E1022, 1994
6. Lacey JM, Wilmore DW: Is glutamine a conditionally essential amino acid? *Nutr Rev* 48:297–309, 1990
7. Smith R: Glutamine metabolism and its physiologic importance. *JPEN* 14:405–445, 1990
8. Kovacevic Z, McGivan JD: Mitochondrial metabolism of glutamine and glutamate and its physiological significance. *Physiol Rev* 63:547–605, 1983
9. Bulus N, Cersosimo E, Ghishan F, Abumrad NN: Physiologic importance of glutamine. *Metabolism* 38(Suppl 1):1–5, 1989
10. Souba WW: Glutamine: A key substrate for the splanchnic bed. *Annu Rev Nutr* 11:285–308, 1991
11. Welbourne TC: Interorgan glutamine flow in metabolic acidosis. *Am J Physiol* 253:F1069–F1076, 1987
12. Souba WW, Klimberg VS, Plumley DA, Salloum RM, Flynn TC, Bland KI, Copeland EM: The role of glutamine in maintaining a healthy gut and supporting the metabolic response to injury and infection. *J Surg Res* 48:383–391, 1990
13. Hall JC, Heel K, McCauley R: Glutamine. *Br J Surg* 83:305–312, 1996
14. Ziegler TR, Szeszycki EE, Estivariz CF, Puckett AB, Leader LM: Glutamine: From basic science to clinical applications. *Nutrition* 12:S68–S70, 1996
15. Jenssen T, Nurjhan N, Perriello G, Bucci A, Toft I, Gerich J: Determination of [^{14}C] glutamine specific activity in plasma. *J Liquid Chromatogr* 17:1337–1348, 1994
16. Darmaun D, D'Amore D, Haymond M: Determination of glutamine and α-ketoglutarate concentration and specific activity in plasma using high-performance liquid chromatography. *J Chromatogr* 620:33–38, 1993
17. Darmaun D, Matthews DE, Bier DM: Glutamine and glutamate kinetics in humans. *Am J Physiol* 251:E117–E126, 1986
18. Perriello G, Jorde R, Nurjhan N, Stumvoll M, Dailey G, Jenssen T, Bier DM, Gerich JE: Estimation of the glucose-alanine-lactate-glutamine cycles in postabsorptive man: Role of skeletal muscle. *Am J Physiol* 269:E443–E450, 1995
19. Garber AJ, Karl IE, Kipnis DM: Alanine and glutamine synthesis and release from skeletal muscle. I. Glycolysis and amino acid release from skeletal muscle. *J Biochem* 251:826–835, 1976
20. Chang T, Goldberg A: The origin of alanine produced in skeletal muscle. *J Biol Chem* 253:3677–3684, 1978
21. Goldberg A, Chang T: Regulation and significance of amino acid metabolism in skeletal muscle. *Fed Proc* 37:2301–2307, 1978
22. Vinay P, Mapes JP, Krebs HA: Fate of glutamine carbon in renal metabolism. *Am J Physiol* 234:F123–F129, 1978
23. Vinay P, Lemieux G, Gougoux A, Halperin ML: Regulation of glutamine metabolism in the dog kidney. *Kidney Int* 29:68–79, 1986
24. Wirthensohn G, Guder WG: Renal substrate metabolism. *Physiol Rev* 66:469–497, 1986
25. Shulman GI, Landau BR: Pathways of glycogen repletion. *Physiol Rev* 72:1019–1035, 1992
26. Askanazi J, Elwyn D, Kinney J, Gump F, Michelson C, Strinchfield F, Fürst P, Vinnars E, Bergström J: Muscle and plasma amino acids after injury: The role of inactivity. *Ann Surg* 188:791–803, 1978
27. Kominz DR, Hough A, Symonds P, Laki K: The amino acid composition of action, myosin, tropomyosin and the meronyosins. *Arch Biochem Biophys* 50:148–159, 1954
28. Wicklmayr M, Rett K, Schwiegelshohn B, Wolfram G, Hailer S, Dietze G: Inhibition of muscular amino acid release by lipid infusion in man. *Eur J Clin Invest* 17:301–305, 1987
29. Pozefsky T, Tancredi RG, Moxley RT, Dupre J, Tobin JD: Effects of brief starvation on muscle amino acid metabolism in nonobese man. *J Clin Invest* 57:444–449, 1976
30. Abumrad NN, Rabin D, Wise KL, Lacy WW: The disposal of an intravenously administered amino acid load across the human forearm. *Metabolism* 31:463–470, 1982
31. Pozefsky T, Walser M: Effect of intraarterial infusion of the ketoanalogue of leucine on amino acid release by forearm muscle. *Metabolism* 26:807–815, 1977
32. Kreider M, Stumvoll M, Meyer C, Overkamp D, Welle S, Gerich J: Steady state and non-steady state measurements of plasma glutamine turnover in humans. *Am J Physiol* 272:E621–E627, 1997
33. Nurjhan N, Bucci A, Perriello G, Stumvoll M, Dailey G, Bier D, Toft I, Jenssen T, Gerich J: Glutamine: A major gluconeogenic precursor and vehicle for interorgan carbon transport in man. *J Clin Invest* 95:272–277, 1995
34. Stumvoll M, Perriello G, Nurjhan N, Bucci A, Welle S, Jansson P, Dailey G, Bier D, Jenssen T, Gerich J: Glutamine and alanine metabolism in NIDDM. *Diabetes* 45:863–868, 1996
35. Gore DC, Jahoor F: Glutamine kinetics in burn patients: Comparison with hormonally induced stress in volunteers. *Arch Surg* 129:1318–1323, 1994
36. Darmaun D, Rongier M, Koziet J, Robert J: Glutamine nitrogen kinetics in insulin-dependent diabetic humans. *Am J Physiol* 261:E713–E718, 1991
37. Hankard R, Goulet O, Ricour C, Rongier M, Colomb V, Darmaun D: Glutamine metabolism in children with short-bowel syndrome: A stable isotope study. *Pediatr Res* 36:202–206, 1994

38. Mauras N, Haymond MW, Darmaun D, Vieira NE, Abrams SA, Yergey AL: Calcium and protein kinetics in prepubertal boys: Positive effects of testosterone. *J Clin Invest* 93:1014–1019, 1994
39. Matthews DE, Pesola G, Campbell RG: Effect of epinephrine on amino acid and energy metabolism in humans. *Am J Physiol* 258:E948–E956, 1990
40. Hankard RG, Haymond MW, Darmaun D: Role of glutamine as a glucose precursor in fasting humans. *Diabetes* 46:1535–1541, 1997
41. Gerich JE: Control of glycaemia. *Baillieres Clin Endocrinol Metab* 7:551–586, 1993
42. Exton J: Gluconeogenesis. *Metabolism* 21:945–990, 1972
43. Landau BR, Wahren J, Chaandramouli V, Schumann WC, Ekberg K, Kalhan SC: Contributions of gluconeogenesis to glucose production in the fasted state. *J Clin Invest* 98:378–385, 1996
44. Mayes PA: Gluconeogenesis and control of the blood glucose, in *Harper's Biochemistry*, edited by Murray RK, Granner DK, Mayes PA, Rodwell VW, East Norwalk, Appleton and Lange, 1993, p 190
45. Kreisberg R, Pennington L, Boshell B: Lactate turnover and gluconeogenesis in obesity: Effect of phenformin. *Diabetes* 19:64–69, 1970
46. Consoli A, Nurjhan N, Reilly JJ Jr, Bier DM, Gerich JE: Contribution of liver and skeletal muscle to alanine and lactate metabolism in man. *Am J Physiol* 259:E677–E685, 1990
47. Bortz WM, Paul P, Haff AC, Holmes WL: Glycerol turnover and oxidation in man. *J Clin Invest* 51:1537–1546, 1972
48. Nurjhan N, Consoli A, Gerich J: Increased lipolysis and its consequences on gluconeogenesis in noninsulin-dependent diabetes mellitus. *J Clin Invest* 89:169–175, 1992
49. Krebs HA: Renal gluconeogenesis. *Adv Enzyme Reg* 1:385–400, 1963
50. Castellino P, Defronzo RA: Glucose metabolism and the kidney. *Semin Nephrol* 10:458–463, 1990
51. Steinberg D: Regulation of carbohydrate metabolism, in *Physiological Basis of Medical Practice*, edited by Satterfield T, Baltimore, Williams & Wilkins, 1991, p 728
52. Felig P, Bergman M: The endocrine pancreas: Diabetes mellitus, in *Endocrinology and Metabolism*, edited by Felig P, Baxter JD, Frohman LA, New York, McGraw-Hill, 1995, p 1107
53. Björkman O, Felig P, Wahren J: The contrasting responses of splanchnic and renal glucose output to gluconeogenic substrates and to hypoglucagonemia in 60-h-fasted humans. *Diabetes* 29:610–616, 1980
54. Björkman O, Felig P: Role of the kidney in the metabolism of fructose in 60-hour fasted humans. *Diabetes* 31:516–520, 1982
55. Brundin T, Wahren J: Renal oxygen consumption, thermogenesis, and amino acid utilization during iv infusion of amino acids in man. *Am J Physiol* 267:E648–E655, 1994
56. Stumvoll M, Overkamp D, Gerich JE: A primer on tracer methods for the study of glucose metabolism in man. *Diabet Nutr Metab* 8:298–314, 1995
57. Stanley W, Gertz E, Wisneski J, Neese R, Morris D, Brooks G: Lactate extraction during net lactate release in legs of humans during exercise. *J Appl Physiol* 60:1116–1120, 1986
58. Kida K, Nakago S, Kamiya F, Toyama Y, Takashi N, Nakagawa H: Renal net glucose release in vivo and its contribution to blood glucose in rats. *J Clin Invest* 62:721–726, 1978
59. McGuinness OP, Fugiwara T, Murrell S, Bracy D, Neal D, O'Connor D, Cherrington AD: Impact of chronic stress hormone infusion on hepatic carbohydrate metabolism in the conscious dog. *Am J Physiol* 265:E314–E322, 1993
60. Cersosimo E, Judd RL, Miles JM: Insulin regulation of renal glucose metabolism in conscious dogs. *J Clin Invest* 93:2584–2589, 1994
61. Stumvoll M, Welle S, Chintalapudi U, Perriello G, Gutierrez O, Gerich J: Uptake and release of glucose by the human kidney: Postabsorptive rates and responses to epinephrine. *J Clin Invest* 96:2528–2533, 1995
62. Stumvoll M, Meyer C, Perriello G, Kreider M, Welle S, Gerich J: Human kidney and liver gluconeogenesis: Evidence for organ substrate selectivity. *Am J Physiol* 274:E817–E826, 1998
63. Stumvoll M, Meyer C, Kreider M, Perriello G, Gerich J: Effects of glucagon on renal and hepatic glutamine gluconeogenesis in normal postabsorptive humans. *Metabolism* 47:1227–1232, 1998
64. Biava C, Grossman A, West M: Ultrastructural observations on renal glycogen in normal and pathologic human kidneys. *Lab Invest* 15:330–356, 1966
65. Guder WG, Ross BD: Enzyme distribution along the nephron. *Kidney Int* 26:101–111, 1984
66. Burch HB, Narins RG, Chu C, Fagioli S, Choi S, McCarthy W, Lowry OH: Distribution along the rat nephron of three enzymes of gluconeogenesis in acidosis and starvation. *Am J Physiol* 235:F246–F253, 1978
67. Stumvoll M, Meyer C, Mitrakou A, Nadkarni V, Gerich J: Renal glucose production and utilization: New aspects in humans. *Diabetologia* 40:749–757, 1997
68. Ross BD, Hems R, Krebs HA: The rate of gluconeogenesis from various precursors in the perfused rat liver. *Biochem J* 102:942–951, 1967
69. Krebs HA, Bennett DAH, Degasquet P, Gascoyne T, Yoshida T: Renal gluconeogenesis the effect of diet on the gluconeogenic capacity of rat kidney cortex slices. *Biochem J* 86:22–28, 1963
70. Egan AR, Black AL: Glutamic acid metabolism in the lactating dairy cow. *J Nutr* 96:450–460, 1968
71. Aikawa T, Matsukata H, Takezawa K, Ishikawa E: Gluconeogenesis and amino acid metabolism. I. Comparison of various precursors for hepatic gluconeogenesis in vivo. *Biochim Biophys Acta* 279:234–244, 1972
72. Wolff J, Bergman E: Gluconeogenesis from plasma amino acids in fed sheep. *Am J Physiol* 223:455–460, 1972
73. Bergman EN, Heitmann RN: Metabolism of amino acids by the gut, liver, kidneys, and peripheral tissues. *FASEB J* 37:1228–1232, 1978
74. Reilly P, Ford E: The effects of different dietary contents of protein on amino acid and glucose production and on the contribution of amino acids to gluconeogenesis in sheep. *Br J Nutr* 26:249–263, 1971
75. Heitmann RN, Bergman EN: Glutamine metabolism, interorgan transport, and glucogenicity in the sheep. *Am J Physiol* 234:E197–E203, 1978
76. Heitmann RN, Bergman EN: Transport of amino acids in whole blood and plasma of sheep. *Am J Physiol* 239:E242–E247, 1980
77. Marliss E, Aoki T, Posefsky T, Mosh A, Cahill G: Muscle and splanchnic glutamine and glutamine metabolism in postabsorptive and starved man. *J Clin Invest* 50:814–817, 1971
78. Nishiitsutsuji-Uwo JM, Ross BD, Krebs HA: Metabolic activities of the isolated perfused rat kidney. *Biochem J* 103:852–862, 1967
79. Krebs H: Glutamine metabolism in the animal body, in *Glutamine: Metabolism, Enzymology and Regulation*, edited by Mora J, Palacios R, New York, Academic Press, 1980, p 319
80. Felig P: The glucose-alanine cycle. *Metabolism* 22:179–207, 1973
81. Felig P: Amino acid metabolism in man. *Annu Rev Biochem* 44:933–955, 1975
82. Rodwell VW: Catabolism of proteins and of amino acid nitrogen, in *Harper's Biochemistry*, edited by Murray RK, Granner DK, Mayes PA, Rodwell VW, East Norwalk, Appleton and Lange, 1993, p 293
83. Felig P, Pozefsky T, Marliss E, Cahill GF Jr: Alanine: Key role in gluconeogenesis. *Science* 167:1003–1004, 1970
84. Esenmo E, Chandramouli V, Schumann WC, Kumaran K, Wahren J, Landau BR: Use of $^{14}CO_2$ in estimating rates of hepatic gluconeogenesis. *Am J Physiol* 263:E36–E41, 1992
85. Owen OE, Reichle FA, Mozzoli MA, Kreulin T, Patel MS, Elfenbein IB, Golsorkhi M, Chang KHY, Rao NS, Sue HS, Boden G: Hepatic, gut, and renal substrate flux rates in patients with hepatic cirrhosis. *J Clin Invest* 68:240–252, 1981
86. Magnusson I, Schumann WC, Bartsch GE, Chandramouli V, Kumaran K, Wahren J, Landau BR: Noninvasive tracing of Krebs cycle metabolism in liver. *J Biochem* 266:6975–6984, 1991
87. Consoli A, Kennedy F, Miles J, Gerich J: Determination of Krebs cycle metabolic carbon exchange in vivo and its use to estimate the individual contributions of gluconeogenesis and gly-

cogenolysis to overall hepatic glucose output in man. *J Clin Invest* 80:1303–1310, 1987
88. KATZ J: Determination of gluconeogenesis in vivo with ^{14}C-labeled substrates. *Am J Physiol* 248:R391–R399, 1985
89. EXTON JH, PARK CR: Control of gluconeogenesis in the liver. *J Biochem* 242:2622–2630, 1967
90. KALOYIANNI M, FREEDLAND RA: Contribution of several amino acids and lactate to gluconeogenesis in hepatocytes isolated from rats fed various diets. *J Nutr* 120:116–122, 1990
91. JOSEPH S, MCGIVAN J: The effect of ammonium chloride and glucagon on the metabolism of glutamine in isolated liver cells from starved rats. *Biochim Biophys Acta* 543:16–28, 1978
92. ROXE DM, SCHREINER GE, PREUSS HG: Regulation of renal gluconeogenesis and ammoniagenesis by physiologic fuels. *Am J Physiol* 225:908–911, 1973
93. HEITMANN RN, BERGMAN EN: Glutamate interconversions and glucogenicity in the sheep. *Am J Physiol* 241:E465–E472, 1981
94. BJÖRKMAN O, ERIKSSON L, NYBERG B, WAHREN J: Gut exchange of glucose and lactate in basal state and after oral glucose ingestion in postabsorptive patients. *Diabetes* 39:747–751, 1990
95. KELLEY D, MITRAKOU A, MARSH H, SCHWENK F, BENN J, SONNENBERG G, ARCHANGELI M, AOKI T, SORENSEN J, BERGER M, SONKSEN P, GERICH J: Skeletal muscle glycolysis, oxidation, and storage of an oral glucose load. *J Clin Invest* 81:1563–1571, 1988
96. GRUBB B: De novo synthesis of alanine by the perfused rat hindlimb. *Am J Physiol* 230:1379–1384, 1976
97. RUDERMAN N, BERGER M: The formation of glutamine and alanine in skeletal muscle. *J Biol Chem* 249:5500–5506, 1974
98. ROBERT J, BIER DM, ZHAO XH, MATTHEWS DE, YOUNG VR: Glucose and insulin effects on de novo amino acid synthesis in young men studied with stable isotope labelled alanine, glycine, leucine and lysine. *Metabolism* 31:1210–1217, 1982
99. PITTS RF, STONE WJ: Renal metabolism of alanine. *J Clin Invest* 46:530–538, 1967
100. CHOCHINOV RH, BOWEN HF, MOORHOUSE JA: Circulating alanine disposal in diabetes mellitus. *Diabetes* 27:420–426, 1978
101. CHIASSON J, ATKINSON R, CHERRINGTON D, KELLER V, SINCLAIR-SMITH B, LACY W, LILJENQUIST J: Effects of fasting on gluconeogenesis from alanine in nondiabetic man. *Diabetes* 28:56–60, 1979
102. MALLETTE LE, EXTON JH, PARK CR: Control of gluconeogenesis from amino acids in the perfused rat liver. *J Biol Chem* 244:5713–5723, 1969
103. CORI C: The glucose-lactate acid cycle and gluconeogenesis. *Curr Topics Cell Reg* 18:377–387, 1981
104. KREISBERG R: Glucose-lactate interrelations in man. *N Engl J Med* 287:132–137, 1972
105. WATERHOUSE C, KEILSON J: The contribution of glucose to alanine metabolism in man. *J Lab Clin Med* 92:803–812, 1978
106. CALDECOURT MA, COX DJ, SUGDEN MC, PALMER TN: Glycolytic origin of alanine formed in rat diaphragm muscle in vitro. *Biochem J* 231:801–804, 1985
107. CORNELL NW, LUND P, KREBS HA: The effect of lysine on gluconeogenesis from lactate in rat hepatocytes. *Biochem J* 142:327–337, 1974
108. KREBS HA, YOSHIDA T: Renal gluconeogenesis. II. The gluconeogenic capacity of the kidney cortex of various species. *Biochem J* 89:398–400, 1963
109. GUDER WG, WIRTHENSOHN G: Metabolism of isolated kidney tubules: Interactions between lactate, glutamine and oleate metabolism. *Eur J Biochem* 99:577–584, 1979
110. DEROSA G, SWICK RW: Metabolic implications of the distribution of the alanine aminotransferase isoenzymes. *J Biol Chem* 250: 7961–7967, 1975
111. FELIG P, WAHREN J, RÄF L: Evidence of interorgan amino acid transport by blood cells in humans. *Proc Natl Acad Sci USA* 70:1775–1779, 1973
112. MILLER BM, CERSOSIMO E, MCRAE J, WILLIAMS PE, LACY WW, ABUMRAD NN: Interorgan relationship of alanine and glutamine during fasting in the conscious dog. *J Surg Res* 35:310–318, 1983
113. AIKAWA T, MATSUTAKA H, YAMAMOTO H, OKUDA T, ISHIKAWA E, KAWANO T, MATSUMURA E: Inter-organal relations and roles of glutamine and alanine in the amino acid metabolism of fasted rats. *J Biochem* 74:1003–1017, 1973
114. WINDMUELLER HG, SPAETH AE: Uptake and metabolism of plasma glutamine by the small intestine. *J Biol Chem* 249:5070–5079, 1974
115. CHRISTENSEN HN: Role of amino acid transport and countertransport in nutrition and metabolism. *Physiol Rev* 70:43–77, 1990
116. SHOTWELL MA, KILBERG MS, OXENDER DL: The regulation of neutral amino acid transport in mammalian cells. *Biochim Biophys Acta* 737:267–284, 1983
117. SIPS HJ, GROEN AK, TAGER JM: Plasma-membrane transport of alanine is rate-limiting for its metabolism in rat-liver parenchymal cells. *FEBS Lett* 119:271–274, 1980
118. KRISTENSEN LO, SESTOFT L, FOLKE M: Concentrative uptake of alanine in hepatocytes from fed and fasted rats. *Am J Physiol* 244:G491–G500, 1983
119. CURTHOYS N, WAITFORD M: Regulation of glutaminase activity and glutamine metabolism. *Annu Rev Nutr* 15:133–159, 1995
120. JOSEPH SK, MCGIVAN JD: The effects of ammonium chloride and bicarbonate on the activity of glutaminase in isolated liver mitochondria. *Biochem J* 176:837–844, 1978
121. PILKIS SJ, GRANNER DK: Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu Rev Physiol* 54:885–909, 1992
122. EXTON JH: Mechanisms of hormonal regulation of hepatic glucose metabolism. *Diabetes Metab Rev* 3:163–183, 1987
123. HERS HG, HUE L: Gluconeogenesis and related aspects of glycolysis. *Annu Rev Biochem* 52:617–653, 1983
124. PILKIS SJ, EL-MAGHRABI MR: Hormonal regulation of hepatic gluconeogenesis and glycolysis. *Annu Rev Biochem* 57:755–783, 1988
125. RIZZA R, MANDARINO L, GERICH J: Dose–response characteristics for the effects of insulin on production and utilization of glucose in man. *Am J Physiol* 240:E630–E639, 1986
126. MEYER C, DOSTOU J, NADKARNI V, GERICH J: Effects of physiological hyperinsulinemia on systemic and renal glucose, glutamine, and free fatty acid metabolism. (abstract) *Diabetes* 47(Suppl 1): A41, 1998
127. LUPIANEZ JA, DILEEPAN KN, WAGLE SR: Interrelationship of somatostatin, insulin and calcium in the control of gluconeogenesis in kidney cortex slices. *Biochem Biophys Res Commun* 90:1153–1158, 1979
128. BROCKMAN R, BERGMAN E, JOO P, MANNS J: Effects of glucagon and insulin on net hepatic metabolism of glucose precursors in sheep. *Am J Physiol* 229:1344–1350, 1975
129. BROCKMAN R, BERGMAN E: Effect of glucagon on plasma alanine and glutamine metabolism and hepatic gluconeogenesis in sheep. *Am J Physiol* 228:1627–1633, 1975
130. BOWMAN RH: Gluconeogenesis in the isolated perfused rat kidney. *J Biol Chem* 245:1604–1612, 1970
131. MOREL F: Sites of hormone action in the mammalian nephron. *Am J Physiol* 240:F159–F164, 1981
132. BATTEZZATI A, SIMONSON DC, LUZI L, MATTHEWS DE: Glucagon depletes circulating glutamine by increasing glutamine uptake and not affecting its release. (abstract) *Diabetes* 45(Suppl 2):253A, 1996
133. BRYLA J, HARRIS EJ, PLUMB JA: The stimulatory effect of glucagon and dibutyryl AMP on ureogenesis and gluconeogenesis in relation to the mitochondrial ATP content. *FEBS Lett* 80:443–448, 1977
134. MITRAKOU A, RYAN C, VENEMAN T, MOKAN M, JENSSEN T, KISS I, DURRANT J, CRYER P, GERICH J: Hierarchy of thresholds for activation of counterregulatory hormone secretion, symptoms, and cerebral dysfunction. *Am J Physiol* 260:E67–E74, 1991
135. KUROKAWA K, MASSRY SG: Evidence for stimulation of renal gluconeogenesis by catecholamines. *J Clin Invest* 52:961–964, 1973
136. SACCA L, VIGORITO C, CICALA M, CORSO G, SHERWIN RS: Role of gluconeogenesis in epinephrine-stimulated hepatic glucose production in humans. *Am J Physiol* 245:E294–E302, 1983
137. CERSOSIMO E, GARLICK P, FERRETTI J: Renal glucose production during insulin-induced hypoglycemia in humans. (abstract) *Diabetes* 47(Suppl 1):A41, 1998
138. MEYER C, DOSTOU J, GERICH J: Important role of the kidney in hypoglycemia counterregulation in humans. (abstract) *Diabetes* 47(Suppl 1):A38, 1998

139. Schoolwerth AC, Smith BC, Culpepper RM: Renal gluconeogenesis. *Miner Electrolyte Metab* 14:347–361, 1988
140. Darmaun D, Matthews DE, Bier DM: Physiological hypercortisolemia increases proteolysis, glutamine, and alanine production. *Am J Physiol* 255:E366–E373, 1988
141. Aber GM, Morris LO, Housley E: Gluconeogenesis by the human kidney. *Nature* 212:1589–1590, 1966
142. Pitts RF, Pilkington LA, MacLeod MB, Leal-Pinto E: Metabolism of glutamine by the intact functioning kidney of the dog. *J Clin Invest* 51:557–565, 1972
143. Alleyne GAO, Roobol A: Renal metabolic processes and acid-base changes. *Med Clin North Am* 59:781–793, 1975
144. Kamm DE, Cahill GF Jr: Effect of acid-base status on renal and hepatic gluconeogenesis in diabetes and fasting. *Am J Physiol* 216:1207–1212, 1969
145. Goodman AD, Fuisz RE, Cahill GF Jr: Renal gluconeogenesis in acidosis, alkalosis, and potassium deficiency: Its possible role in regulation of renal ammonia production. *J Clin Invest* 45:612–619, 1966
146. Nissim I, Yudkoff M, Segal S: Metabolic fate of glutamate carbon in rat renal tubules. *Biochem J* 241:361–370, 1987
147. Schoolwerth AC, Deboer P, Moorman AF, Lamers WH: Time course of changes in mRNAs for enzymes of glutamine metabolism in kidney during metabolic acidosis. *Contrib Nephrol* 110: 127–132, 1994
148. Welbourne TC, Phromphetcharat V: Renal glutamine metabolism and hydrogen ion homeostasis, in *Glutamine Metabolism in Mammalian Tissue*, edited by Häussinger D, Sies H, Berlin/Heidelberg, Springer-Verlag, 1984, p 161
149. Addae SK, Lotspeich WD: Relationship between glutamine utilization and production in metabolic acidosis. *Am J Physiol* 215:269–277, 1968
150. Fine A: The effects of chronic metabolic acidosis on liver and muscle glutamine metabolism in the dog in vivo. *Biochem J* 202:271–273, 1982
151. Cersosimo E, Williams P, O'Donovan D, Lacy D, Abumrad N: Role of acidosis in regulating hepatic nitrogen metabolism during fasting in conscious dog. *Am J Physiol* 252:E313–E319, 1987
152. Tamarappoo B, Joshi S, Welbourne T: Interorgan glutamine flow regulation in metabolic acidosis. *Miner Electrolyte Metab* 16:322–330, 1990
153. Schröck H, Goldstein L: Interorgan relationship for glutamine metabolism in normal and acidotic rats. *Am J Physiol* 240:E519–E525, 1981
154. Sies H, Häussinger D: Hepatic glutamine and ammonia metabolism, in *Glutamine Metabolism in Mammalian Tissues*, edited by Häussinger D, Sies H, Berlin/Heidelberg, Springer-Verlag, 1984, p 78
155. Guder WG, Häussinger D, Gerok W: Renal and hepatic nitrogen metabolism in systemic acid base regulation. *J Clin Chem Clin Biochem* 25:457–466, 1987
156. Hetenyi G: Correction for the metabolic exchange of ^{14}C for ^{12}C atoms in the pathway of gluconeogenesis in vivo. *Fed Proc* 41:104–109, 1982
157. Kelleher JK: Gluconeogenesis from labelled carbon: estimating isotope dilution. *Am J Physiol* 250:E296–E305, 1986
158. Lavoinne A, Baquet A, Hue L: Stimulation of glycogen synthesis and lipogenesis by glutamine in isolated rat hepatocytes. *Biochem J* 248:429–437, 1987
159. Cersosimo E, Williams P, Hoxworth B, Lacy W, Abumrad N: Glutamine blocks lipolysis and ketogenesis of fasting. *Am J Physiol* 250:E248–E252, 1986
160. Wu G, Thompson JR: The effect of glutamine on protein turnover in chick skeletal muscle in vitro. *Biochem J* 265:593–598, 1990
161. Pösö A, Schworer C, Mortimore G: Acceleration of proteolysis in perfused rat liver by deletion of glucogenic amino acids: Regulatory role of glutamine. *Biochem Biophys Res Commun* 107:1433–1439, 1982
162. Seglen PO, Gordon PB, Poli A: Amino acid inhibition of the autophagic/lysosomal pathway of protein degradation in isolated rat hepatocytes. *Biochim Biophys Acta* 630:103–118, 1980
163. MacLennan P, Smith K, Weryk B, Watt P, Rennie M: Inhibition of protein breakdown by glutamine in perfused rat skeletal muscle. *FEBS Lett* 237:133–136, 1988
164. Jepson M, Bates P, Broadbent P, Pell J, Millward D: Relationship between glutamine concentration and protein synthesis in rat skeletal muscle. *Am J Physiol* 255:E166–E172, 1988
165. Perriello G, Nurjhan N, Stumvoll M, Bucci A, Welle S, Dailey G, Bier DM, Toft I, Jenssen TG, Gerich JE: Regulation of gluconeogenesis by glutamine in normal postabsorptive humans. *Am J Physiol* 272:E437–E445, 1997
166. Hankard RG, Darmaun D, Sager BK, D'Amore D, Parsons WR, Haymond M: Response of glutamine metabolism to exogenous glutamine in humans. *Am J Physiol* 269:E663–E670, 1995
167. Jenssen T, Nurjhan N, Consoli A, Gerich JE: Dose-response effects of lactate infusions on gluconeogenesis from lactate in normal man. *Eur J Clin Invest* 23:448–454, 1993
168. Herrera MG, Kamm D, Ruderman N, Cahill GF Jr: Non-hormonal factors in the control of gluconeogenesis. *Adv Enzyme Regul* 4:225–235, 1966
169. Peters HH, Stumpf B, Hamm HH, Graf B, Boie-Nath A, Stiller G, Seubert W: Regulation of phosphoenolpyruvate carboxykinase by glutamine and ATP as possible control mechanisms of renal gluconeogenesis. *Curr Prob Clin Biochem* 6:336–345, 1976
170. Mojena M, Bosca L, Hue L: Effect of glutamine on fructose 2,6-biphosphate and on glucose metabolism in HeLa cells and in chicken-embryo fibroblasts. *Biochem J* 232:521–527, 1985
171. Pilkis SJ, El-Maghrabi MR, Claus TH: Fructose-2,6-bisphosphate in control of hepatic gluconeogenesis. *Diabetes* 13:582–599, 1990
172. Friedrichs D: On the stimulation of gluconeogenesis by L-lysine in isolated rat kidney cortex tubules. *Biochim Biophys Acta* 392:255–270, 1975
173. Katz J, Golden S, Wals PA: Stimulation of hepatic glycogen synthesis by amino acids. *Proc Natl Acad Sci USA* 73:3433–3437, 1976
174. Katz J, Golden S, Wals PA: Glycogen synthesis by rat hepatocytes. *Biochem J* 180:389–402, 1979
175. Rognstad R: Possible role for carbamyl phosphate in the control of liver glycogen synthesis. *Biochem Biophys Res Commun* 130:229–233, 1985
176. Mouterde O, Claeyssens S, Chedeville A, Lavoinne A: Glutamine is a good substrate for glycogen synthesis in isolated hepatocytes from 72h-starved rats, but not from 24 h- or 48 h-starved rats. *Biochem J* 288:795–799, 1992
177. Radziuk J: Hepatic glycogen in humans. II. Gluconeogenetic formation after oral and intravenous glucose. *Am J Physiol* 257:E158–E169, 1989
178. Radziuk J: Hepatic glycogen in humans. I. Direct formation after oral and intravenous glucose or after a 24-h fast. *Am J Physiol* 257:E145–E157, 1989
179. Baquet A, Hue L, Meijer AJ, Van Woerkom GM, Plomp PJAM: Swelling of rat hepatocytes stimulates glycogen synthesis. *J Biol Chem* 265:955–959, 1990
180. Plomp PJAM, Boon L, Caro LHP, Van Woerkom GM, Meijer AJ: Stimulation of glycogen synthesis in hepatocytes by added amino acids is related to the total intracellular content of amino acids. *Eur J Biochem* 191:237–243, 1990
181. Baquet A, Lavoinne A, Hue L: Comparison of the effects of various amino acids on glycogen synthesis, lipogenesis and ketogenesis in isolated rat hepatocytes. *Biochem J* 273:57–62, 1991
182. Bode AM, Foster JD, Nordlie RC: Glyconeogenesis from L-proline involves metabolite inhibition of the glucose-6-phosphatase system. *J Biol Chem* 267:2860–2863, 1992
183. Bode A, Nordlie R: Reciprocal effects of proline and glutamine on glycogenesis from glucose and ureagenesis in isolated, perfused rat livers. *J Biol Chem* 268:16298–16301, 1993
184. Scislowski PWD, Niblock A, Lindsay Y, Weryk B, Watt PW, Rennie MJ: Glutamine stimulates glycogen synthesis in skeletal muscle. (abstract) *Clin Nutr* 8:P80, 1989
185. Varnier M, Leese GP, Thompson J, Rennie MJ: Stimulatory effect of glutamine on glycogen accumulation in human skeletal muscle. *Am J Physiol* 269:E309–E315, 1995
186. Szabo A, Kenesei E, Miltenyi M, Szücs L, Nagy I: Changes in

plasma and urinary amino acid levels during diabetic ketoacidosis in children. *Diabetes Res Clin Pract* 12:91–97, 1991
187. Wahren J, Felig P, Hagenfeldt L: Effect of protein ingestion on splanchnic and leg metabolism in normal man and in patients with diabetes mellitus. *J Clin Invest* 57:987–999, 1976
188. Felig P, Wahren J, Karl I, Cerasi E, Luft R, Kipnis DM: Glutamine and glutamine metabolism in normal and diabetic subjects. *Diabetes* 22:573–576, 1973
189. Luzi L, Petrides AS, Defronzo RA: Different sensitivity of glucose and amino acid metabolism to insulin in NIDDM. *Diabetes* 42:1868–1877, 1993
190. Puhakainen I, Koivisto V, Yki-Jarvinen H: Lipolysis and gluconeogenesis from glycerol are increased in patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 75:789–794, 1992
191. Watford M, Erbelding EJ, Smith EM: The regulation of glutamine and ketone-body metabolism in the small intestine of the long-term (40-day) streptozotocin-diabetic rat. *Biochem J* 242:61–68, 1987
192. Brosnan J, Man K, Hall D, Colbourne S, Brosnan M: Interorgan metabolism of amino acids in the streptozotocin-diabetic ketoacidotic rat. *Am J Physiol* 244:E151–E158, 1983
193. Consoli A, Nurjhan N, Reilly J, Bier D, Gerich J: Mechanism of increased gluconeogenesis in noninsulin dependent diabetes mellitus: Role of alterations in systemic, hepatic and muscle lactate and alanine metabolism. *J Clin Invest* 86:2038–2045, 1990
194. Ardawi M: Glutamine and ketone body metabolism in the gut of streptozotocin-diabetic rats. *Biochem J* 249:565–572, 1988
195. Lemieux G, Achkar M, Vinay P, Gougoux A: Characteristics of ammoniagenesis and gluconeogenesis by the diabetic kidney: In vitro studies in the rat. *Contrib Nephrol* 31:23–28, 1982
196. Lemieux G, Aranda MR, Fournel P, Lemieux C: Renal enzymes during experimental diabetes mellitus in the rat: Role of insulin, carbohydrate metabolism, and ketoacidosis. *Can J Physiol Pharmacol* 62:70–75, 1984
197. Elliott K, Pogson C: The effects of starvation and experimental diabetes on phosphoenolpyruvate carboxykinase in the guinea pig. *Biochem J* 164:357–361, 1977
198. Kang S, Fears R, Noirot S, Mbanga J, Yudkin J: Changes in metabolism of rat kidney and liver caused by experimental diabetes and by dietary sucrose. *Diabetologia* 22:285–288, 1982
199. Mitrakou A, Plantanisiotis D, Vlachos L, Mourikis D, Nadkarni V, Meyer C, Chintalapudi U, Gutierrez O, Kreider M, Gerich J, Raptis S: Increased renal glucose production in insulin dependent diabetes (IDDM): Contribution to systemic glucose appearance and effect of insulin repletion. (abstract) *Diabetes* 45(Suppl 2):33A, 1996
200. Lundholm K, Bennegard K, Zachrisson H, Lundgren F, Eden E, Möller-Loswick A: Transport kinetics of amino acids across the resting human leg. *J Clin Invest* 80:763–771, 1987
201. Elia M, Folmer P, Schlatman A, Goren A, Austin S: Amino acid metabolism in muscle and in whole body of man before and after ingestion of a single mixed meal. *Am J Clin Nutr* 49:1203–1210, 1989
202. Elia M, Neale G, Livesey G: Alanine and glutamine release from the human forearm: Effects of glucose administration. *Clin Sci* 69:123–133, 1985
203. Elia M, Livesey G: Effects of ingested steak and infused leucine on forearm metabolism in man and the fate of the carbon skeletons and amino groups of branched-chain amino acids. *Clin Sci* 64:517–526, 1983
204. Sandler MP, Robinson RP, Rabin D, Lacy WW, Abumrad NN: The effects of thyroid hormones on gluconeogenesis and forearm metabolism in man. *J Clin Endocrinol Metab* 56:479–485, 1983
205. Abumrad N, Robinson R, Gooch B, Lacy W: The effect of leucine infusion on substrate flux across the human forearm. *J Surg Res* 32:453–463, 1982
206. Gelfand RA, Glickman MG, Jacob R, Sherwin RS, Defronzo RA: Removal of infused amino acids by splanchnic and leg tissues in man. *Am J Physiol* 250:E407–E413, 1986
207. Morrison WL, Gibson JNA, Jung RT, Rennie MJ: Skeletal muscle and whole body protein turnover in thyroid disease. *Eur J Clin Invest* 18:62–68, 1988
208. Aoki TT, Brennan MF, Fitzpatrick GF, Knight DC: Leucine meal increases glutamine and total nitrogen release from forearm muscle. *J Clin Invest* 68:1522–1528, 1981
209. Lochs H, Roth E, Gasic S, Hubl W, Morse EL, Adibi SA: Splanchnic, renal and muscle clearance of alanylglutamine in man and organ fluxes of alanine and glutamine when infused in free dual peptide forms. *Metabolism* 39:833–836, 1990
210. Hagenfeldt L, Eriksson S, Wahren J: Influence of leucine on arterial concentrations and regional exchange of amino acids in healthy subjects. *Clin Sci* 59:173–181, 1980
211. Aoki TT, Brennan MR, Müller WA, Soeldner JS, Alpert JS, Salt SB, Kaufmann RL, Tan MH, Cahill JR: Amino acid levels across normal forearm muscle and splanchnic bed after a protein meal. *Am J Clin Nutr* 29:340–350, 1976
212. Assan R, Marre M, Gormley M: The amino acid-induced secretion of glucagon, in *Glucagon II*, edited by Lefebvre P, New York, Springer-Verlag, 1983, p 19
213. Ratheiser K, Komjati M, Gasic S, Bratusch-Marrain P, Walhouse W: Effect of stress hormones on transplanchic balance of exogenous amino acids in healthy man. *Metabolism* 40:1298–1304, 1991
214. Tizianello A, Deferrari G, Garibotto G, Gurreiri G: Effects of chronic renal insufficiency and metabolic acidosis on glutamine metabolism in man. *Clin Sci Mol Med* 55:391–397, 1978
215. Souba W, Herskowitz K, Klimberg V, Salboum R, Plumley D, Flynn T, Copeland E: The effects of sepsis and endotoxemia on gut glutamine metabolism. *Ann Surg* 211:543–549, 1990
216. Owen OE, Robinson RR: Amino acid extraction and ammonia metabolism by the human kidney during the prolonged administration of ammonium chloride. *J Clin Invest* 42:263–276, 1963
217. Plumley DA, Souba WW, Hautamaki RD, Martin TD, Flynn TC, Rout WR, Copeland EM: Accelerated lung amino acid release in hyperdynamic septic surgical patients. *Arch Surg* 125:57–61, 1990
218. Frayn KN, Khan K, Coppack SW, Elia M: Amino acid metabolism in human subcutaneous tissue in vivo. *Clin Sci* 80:471–474, 1990